




**Data Evaluation Report on the Tier II Data Requirement Waiver Request for HeiQ AGS-20**  
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**Tier II Data Requirements:** Listed in the EPA Memorandum “Updated Data Requirements for the conditional registration of HeiQ AGS-20” of December 11, 2014.

**Decision:** Waiver request is denied. The registrant provided comments on the Agency’s conclusions of the findings of the Tier I dissolution study (MRIDs 492302401, 49327301-submitted originally and reviewed originally under MRID 49165501), the Tier I leaching study (MRID 49141001 [corrected citation, MRID 49144001 was cited in waiver request]), and an extension to the leaching study (MRID 49496601 [an open literature study by Mitrano et al., 2014]). The waiver request does not consist of submittal of any new data to the previous submitted studies, but consists of comments on the interpretation of the study results by the Agency and the opinion that the Agency did not consider the potential for secondary formation (i.e., natural formation) of silver nanoparticles rather than the release of the manufactured silver nanoparticles (MSNPs) in AGS-20 as the basis for the detected silver nanoparticles in the leaching study. Essentially the same waiver request which included the submittal of the Mitrano et al., 2014 study was submitted previously under MRID 49496601, and was denied by the agency (USEPA Memorandum June 11, 2015). A summary statement of the registrant comments in this waiver request (MRID 49710102) and the Agency’s response to each is provided below.

#### **Tier I Dissolution Study**

**Registrant Comment:** Tier I dissolution study (MRIDs [49165501,] 49203401, 49327301) shows AGS-20 releases ionic silver only, no silver nanoparticles were detected in the study therefore the study results do not trigger the conditionally required Tier II data requirements based on a lack of release of the manufactured silver nanoparticles (MSNPs) from AGS-20 complex.

**Agency Response:** This discussion by the registrant was very narrowly focused on the release of the MSNPs and also does not point out some of the limitations of the study discussed by the Agency in its DER of this study regarding concluding that no MSNPs were released and other issues that were documented by the study authors. The trigger and potential test material for Tier II testing in addition to the release of MSNPs also includes the AGS-20 complex and its potential release and fate in the environment (Figure 2A, HeiQ AGS-20 Decision Document<sup>1</sup>), as reiterated by the registrant themselves in the waiver request (Section 4), and in the study report (Section 2.1 Purpose of the study). There are potential pathways for release of the MSNPs, such as the agitation clothing will undergo during

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<sup>1</sup> USEPA. 2011 Decision Document. Conditional Registration of HeiQ AGS-20 as a Material Preservative in Textiles. December 1, 2011. EPA-HQ-OPP-2009-1012-0064 <https://www.regulations.gov/document?D=EPA-HQ-OPP-2009-1012-0064>

washing or abrasion and mechanical shear with sediment and soil particles if the AGS-20 complex reaches the environment, which the dissolution kinetics study alone does not address, therefore the Agency considered results of the Tier I dissolution kinetic study and leaching study together in determining the need for Tier II data requirements as designated in the decision tree in Figure 2A of the HeiQ AGS-20 Decision Document.

The dissolution kinetics study was in theory not only to provide information about the release and dissolution of MSNPs from AGS-20 under environmentally relevant conditions but was also to provide information about the overall stability of the AGS-20 complex and therefore its likelihood to reach the environment, if released. While the dissolution kinetic study had issues which limits the use of the data quantitatively in an exposure assessment, the results clearly showed a relative trend that under natural environmental conditions the AGS-20 complex would be expected to be sufficiently stable to reach and persist in natural environments with some of its surface MSNPs undissolved. For example, in distilled water enough silver was released from AGS-20 to result in a mean maximum of 180 µg/L of silver in the <0.45 µm filtrate whereas the same amount of AGS-20 only resulted in a mean maximum release of 70 µg/L of silver in river water and about 90 µg/L of silver in moderately hard reconstituted water (MHRW) over 40 days. These relative differences provides evidence that there were MSNPs in the AGS-20 complex which could potentially have released silver but were stable to dissolution under environmental natural conditions. The issues with the dissolution kinetic study are as follows:

- The report lacks a complete chemical characterization of the distilled water, modified hard water, and river water. This is especially important for conducting any chemical speciation modeling, and understanding TEM-EDX results.
- There was a lack of full characterization of the actual starting materials at a level that is consistent with the data available through OECD for NM300.
- Documented poor recovery (e.g., mean recovery in the <0.45 µm filtrate silver nitrate moderately hard reconstituted water (MHRW) and river water controls.
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  - The adequacy of the filtering methods were not demonstrated for the tested materials – as such much of the material could have been collected on the filter material include materials that would in theory pass through. A way to demonstrate the method would be to take TEM-EDX images before and after filtering and looking at the distribution and composition of the particles.
- Lack of mass balance (e.g., total silver in unfiltered solution not quantified so total release of silver and loss of silver to sorption by containers not quantified).
  - Part of the poor recovery was speculated by the authors as due to the use of containers that sorbed silver (e.g., Section 4.7 of study report “ of the silver is lost, either to the walls of the bottles...” ). Containers that sorb the test material should not have been used, and without a sorption study for the storage bottles it is impossible to precisely define the reason(s) for loss.
  - Other factors may have been precipitation or nanoparticles sticking to the brown bottle, aggregation, etc.
  - Is is not clear if the released silver is in in the form of Ag<sup>0</sup>, Ag<sup>+</sup>, or Ag-precipitate.
- Unexplained drop in pH in the distilled water.
- Potential low level silver contamination issues which may have obscured detection of low levels of released MSNPs as implicated by the following weight-of-evidence:
  - silver contamination in background blanks;
  - significantly higher silver concentrations detected in all 0.1 µm blank filtrates as compared to the mass of silver measured in the 0.45 µm blank filtrates for all media; and
  - inconsistency in sorption loss of ionic silver to filters within this study and as compared to other studies using the same filter types and inconsistency in how results are interpreted in light of this expected “sorption” loss.

- AGS-20 results generally did not exhibit consistent or progressive loss of silver due to sorption of ionic silver on the order observed in the silver nitrate controls, which the study report described as the typical loss rate for these filters from both the results of the silver nitrate controls and in reference to other studies using these same type of filters—*“In DI water most of the Ag<sup>+</sup> passed through all filters, the loss in each filter was only around 5%...Some loss of Ag is therefore observed in all samples, however, all filters behaved in more or less the same way, even the 10 kDa filter which has been found to adsorb more Ag than the other filters in previous studies”*.
  - Instead of having lower concentrations of silver in the 10 kDa filtrate as seen in the silver nitrate recoveries for all the media due to the “sorption loss to the filter”, the AGS-20 results actually show mean 10 kDa filtrate with higher silver concentrations than the mean <0.45 filtrate in some cases (Figure 3 of study report), and in most cases mean silver concentrations in 10 kDa filtrate are reported as greater than or equal to that of the <0.1 µm filtrate (Figures 3, 4 and 5 of study report). Because the descriptive statistics are presented as independent samples rather than dependent samples, the measure of within-subject variability may obscure differences (see discussion of statistical handling of results, and lack of independent statistical verification in paragraph following the proceeding two bullets).
  - Textile leaching study (MRID 49141001) silver nitrate pure water and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) recovery samples exhibited on average no loss and about a 4% loss, respectively, passing through 0.45 µm filter, and about a 3% and 7% loss, respectively, to the 0.10 µm filter (Section 5.1 of study). The 10 kDa filter loss of dissolved silver in these solutions is identified in the report as 40 to 60%.
- In addition to the possible contamination/lack of filter sorption loss another issue which potentially limits the ability to detect the presence of particles is the lack of accounting for the dependency of the observational unit (e.g., concentrations in replicate 1 filtrate series are not independent of each other, etc.) for estimates of variability. Descriptive statistics were provided as though the filtrate concentrations in an observational unit were independent (i.e., mean 10 kDa filtrate results versus mean 0.1 µm filtrate results) rather than as dependent observational units (e.g., mean [0.1 µm filtrate - 10 kDa filtrate]), which results in higher within-subject variability (this was not previously noted in the DER)<sup>2</sup>, which reduces the ability to detect differences. Raw data was not provided, so an independent statistical analysis of the data considering this dependency could not be performed.

As the data is presented in figures in the report, it **suggests** no MSNPs were released. However, as identified in the discussion above about issues with the study, there is a lack of confidence in this conclusion. This was stated in the DER *“...the data and results are mired in difficulties to unambiguously analyze and interpret results...”*, and resulted in the classification of the study as “Supplemental”.

### **Tier I Leaching Study Interpretation**

**Registrant Comment:** Agency is assuming the silver nanoparticles detected in the Tier I leaching study (MRID 4914[1]001) from AGS-20 surface treated and incorporated treated textiles were due to a direct release of MSNPs from AGS-20, but the Agency should instead identify these as likely due to daughter or secondary formation of silver nanoparticles from ionic silver based on the silver nitrate positive control findings and a 2014 study by Mitrano et al. (MRID 49496601) which looks at the role of other silver treatments in the production of silver nanoparticles.

**Agency Response:** The Agency disagrees that it did not consider these possibilities in the determination that the

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<sup>2</sup> Raw data was not provided with the study which would have allowed an independent statistical analysis of the paired differences (not mentioned in the DER).

textile leaching study with AGS-20 demonstrated release of MSNPs, rather than secondary formation of silver nanoparticles from ionic silver. The Agency considered all the results of both the silver nitrate and OECD standard reference material (NM300) in its consideration of the evidence that neither AGS-20 or its MSNPs are released. Given the limitation of the study and the ability to determine the mechanism of formation or release of silver nanoparticles, additional characterization would be needed on the starting materials as well as materials observed in wash waters to better understand their similarities or differences. More detailed discussion of all the evidence considered in the Agency's decision is provided in the following bullets.

- The adequacy of the filtering methods were not demonstrated for the tested materials and the materials detected in the wash water. While it was speculated that much of the material collected on the filter material includes materials that would in theory pass through a way to demonstrate the filtering method would be appropriate for the purpose of the study was to take TEM-EDX images before and after filtering and looking at the distribution and composition of the particles on the filter.
- No positive control was conducted at the same levels of silver content actually released by the textile treatments. This is important in providing weight to whether the secondary formation mechanism is the primary reason for detection of silver nanoparticles versus release of MSNPs.
  - Not all the MSNPs in AGS-20 are available for dissolution or are vulnerable to sheer. From the dissolution kinetics study about 36% of the total silver in HeiQ AGS-20 was estimated to be available for dissolution (study had some problems so there is large degree of uncertainty in the actual value).
  - The 1,100 µg Ag/L level in the positive control was selected to represent if 100% of the silver was released from the surface textile treatments, which it did not.
  - The silver nitrate positive control nominal total silver content in washing liquid of 1,100 µg Ag/L is 100 times higher than the total silver released in the HeiQ AGS-20 incorporated treatment textile wash water, 31.4 times higher than the NM300 surface treated textile wash water, and almost 10 times higher than the HeiQ AGS-20 surface treatment textile wash water.
- Surfactant results with the NM300 MSNP reference material in the recovery portion of the experiment, and the washing liquid positive controls indicate that the surfactants present in washing liquid stabilized existing MSNPs. This provides evidence that it is possible under study conditions for the MSNPs of HeiQ AGS-20 to also be stabilized by the surfactants present in the detergent used in the study.
  - To obtain a good filtrate recovery for the OECD MSNP reference material (NM300), the study included a recovery experiment where a surfactant (SDS) at 0.1 g/L was used to stabilize and maintain the reference material as dispersed MSNPs, at a loading of 400 µg Ag/L. The use of the surfactant was successful with recoveries through the 0.45 and 0.1 µm filters of 97 and 91.4%, with only 2.8% of the MSNP material recovered as dissolved (10 kDa filtrate). The study authors state *"...the washing liquid contains high concentrations of surfactants, this experiment is therefore more suitable to investigate the behavior of nanoparticles in washing liquid...The recovery was therefore good, indicating that the method of using filtration to size fractionate the particles was functioning well under the chosen conditions."* Results document and implies that in the presence of surfactants such as that in the washing detergent study, any MSNP particles released would for the most part be stable and recoverable per the study recovery documentation.
    - Further support for MSNPs not dissolving and reforming particles is the Scanning Transmission Electron Microscopy ([S]TEM) of the OECD standard reference MSNPs in SDS as stated in the study report *"These particles are clearly still mainly metallic silver, consistent with the NM300 reference material."*
  - Similarly the NM300 OECD standard reference washing liquid positive control at 1,100 µg Ag/L, which is almost three times the amount of silver released into washing liquid from any of the NM300 or AGS-20 textile treatments, documented that the *"...fraction of NM300 that was not retained by the solids in the*

*washing liquids (zeolites) were still present as single nanoparticulate Ag that passed well the 0.1 µm filters...” as stated in the report.*

- The Agency disagrees that the washing detergent positive control silver nitrate results provided definitive proof that silver nanoparticles detected in the AGS-20 portion of the washing detergent study were attributable to secondary formation rather than release of MSNPs from AGS-20. Specific details for this conclusion are discussed in detail in the following bullets.
  - As discussed in the DER for this study and as stated in Table 13 of the study report, no silver particles were observed by electron microscopy in the silver nitrate positive control (1,100 µg Ag/L) where the wash liquid, just like in the textile portion of the study, was not filtered through a 0.45 µm filter prior to introduction of the silver nitrate.
    - The silver nitrate positive control nominal total silver content in washing liquid of 1,100 µg Ag/L is 100 times higher than the total silver released in the HeiQ AGS-20 incorporated treatment textile wash water, 31.4 times higher than the NM300 surface treated textile wash water, and almost 10 times higher than the HeiQ AGS-20 surface treatment textile wash water.
    - Given the limitation of the study and the ability to determine the mechanism of formation or release of silver nanoparticles, additional characterization would be needed on the starting materials as well as materials observed in wash waters to better understand their similarities or differences.
  - The Agency did not consider that the detection of nanoparticles in the silver nitrate “positive” control treatment at a 10 times lower silver concentration (100 µg Ag/L) and where the washing liquid was filtered through a 0.45 µm filter prior to introduction of the silver nitrate was representative of the study conditions with NM300 or HeiQ AGS-20 as none of the washing liquid in the textile treatment portion of the study was passed through a 0.45 µm filter prior to introduction of the treated textiles.
  - The size fractionation pattern in the silver nitrate and OECD reference MSNP positive controls are different. All the silver nanoparticles in the OECD MSNP reference material did not dissolve, as stated in the study report “...fraction of NM300 that was not retained by the solids in the washing liquids (zeolites) were still present as single nanoparticulate Ag that passed well the 0.1 µm filters.” This provides evidence that the nanoparticles present in the NM300 and HeiQ AGS-20 textile treatment wash water cannot be eliminated as due to MSNPs rather than solely to the mechanism of dissolution and reformation of dissolved silver.
  - The “natural” nanoparticles were not characterized as compared to HeiQ. Characterization of the natural nanoparticles should have included physical properties (particle size distribution, surface area) and a complete elemental composition.
  - The TEM-EDX images available are very hard to read and not all the data was presented. For example, EDX peaks are not identified in some of the images and the scale of the TEM image is not provided.
  - A histogram of the particle size distribution should also be provided. This will provide additional information the mechanism (release of formation) by which silver nanoparticles are observed in the wash water.

Note this study also did not provide raw data to allow an independent statistical analysis and the descriptive statistics like that of the dissolution kinetic study suffer from handling of the data as independent observation units rather than dependent.

#### **Consideration of the Formation of Silver Nanoparticles from Ionic Silver**

**Registrant Comment:** Agency did not provide a substantive analysis of the Mitrano et al (2014) study (MRID 49496601), which extends the textile washing detergent study to other conventional silver treatments (silver nitrate, silver chloride, silver zeolites and bulk silver fibers (X-Static)) and provides insight into the significance of ionic silver to the formation of silver nanoparticles under identical conditions and methods as

the existing washing detergent leaching study. This information provides support that the detected silver nanoparticles in the AGS-20 washing leaching study are attributable to secondary transformation from dissolved ionic silver released from AGS-20 and not the actual MSNP from AGS-20.

**Agency Response:** The Agency agrees that it did not fill in a DER specifically for the Mitrano et al. (2014) study contained within the waiver request (MRID 49496601). A brief statement within the waiver response indicated that the additional data on the additional silver treatments contained within the Mitrano et al. (2014) study did not change the Agency's interpretation of the findings from the AGS-20 leaching and dissolution studies submitted to the Agency regarding the release of MSNPs and AGS-20. While a detailed DER was not completed, the Agency stands by its determination that the information on AGS-20, NM300 and other silver active ingredients contained in the Mitrano et al. (2014) study does not negate or provide evidence that eliminates the findings of the leaching and dissolution study (MRIDs 49141001, 49165501, 492302401, 49327301) submitted to the Agency where the nanoparticles present in the NM300 and HeiQ AGS-20 textile treatment wash water cannot be eliminated as due to MSNPs rather than solely to the mechanism of dissolution and reformation of dissolved silver. The Agency is not saying that nanoparticles of silver cannot also form naturally in the environment from ionic silver under the conditions of the textile laundry study, but that also does not mean that their toxicity and fate are exactly the same as the MSNP. The FIFRA Scientific Advisory Panel (SAP) meeting in November, 2009 on assessing the hazard of and exposure to nanosilver active ingredients cautioned about extrapolating from one nanosilver formulation to another when assessing hazards because differences in particle formulation are likely to affect biological activity, among other things. A major issue with this article, as it was with the submitted laundering study, is the lack of characterization of the "natural" nanoparticles as compared to HeiQ silver nanoparticles. Characterization should include physical properties (particle size distribution, surface area) and a complete elemental composition should be done on the natural nanoparticles and how they are similar or dissimilar to the HeiQ product.